A New Triterpenoid Saponin from *Isolatocereus dumortieri*

Kaoru Kinoshita,[†] Kiyotaka Koyama,[†] Kunio Takahashi,*[,†] Norio Kondo,[‡] and Hiroshi Yuasa[‡]

Department of Pharmacognosy and Phytochemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose-shi, Tokyo 204-8588, Japan, and Research Institute of Evolutionary Biology, 2-4-28, Kamiyoga, Setagaya-ku, Tokyo 158-0098, Japan

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A new triterpenoid saponin, named dumortierinoside A, was isolated from *Isolatocereus dumortieri*. The structure was determined as dumortierigenin 3-*O*-α-L-rhamnopyranosyl (1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucuronopyranoside (**1**) on the basis of NMR and mass spectroscopy.

In our previous studies on triterpene sapogenins of several cacti, we have reported some known and new compounds.[1−3] Some triterpene sapogenins from cacti showed antinociceptive activities[4] and antitumor promotion actions.[5] Djerasii and co-workers studied triterpenoid sapogenins of cacti and reported the structure of dumortierigenin from *Lemaireocereus dumortieri* Br. & R. (=*Isolatocereus dumortieri* Backbg.) in 1956.[6] From the same cactus, two triterpene sapogenins, dumortierigenin and a new compound, pachanol D, possessing a new skeletal type named pachanane, were isolated in our course of study.[4] This report describes the isolation and characterization of a new saponin named dumortierinoside A, from the MeOH extract of the cactus.

Dry *I. dumortieri* was extracted with $CHCl_3$ and then repeatedly with MeOH. The MeOH extract was subjected to column chromatography on Si gel to afford a new triterpenoid saponin, which has been named dumortierinoside A (**1**).

Dumortierinoside A (**1**), a colorless powder, had a molecular formula of $C_{48}H_{74}O_{19}$, which was determined from its negative ion HRFABMS and confirmed by ^{13}C NMR and DEPT analysis. The IR spectrum of **1** shows absorptions at 3400 cm^{-1} (hydroxyl) and 1760 cm^{-1} (five-membered lactone). The ^{13}C NMR and DEPT spectra of **1** allowed assignment of 30 of the 48 carbon signals to the aglycone part and 18 to the sugar moiety. The ^1H and ^{13}C NMR spectra of **1** (measured at 60 °C) suggested the presence of three sugar residues, as evidenced by three anomeric carbon signals at *δ* 101.2, 101.8, and 104.5. A carbonyl carbon at *δ* 173.0 suggested the presence of a uronic acid. Acid hydrolysis in 3.5% HCl for 2.5 h at 110 °C afforded dumortierigenin, identified by TLC and comparison with the published spectral data,[2] and three sugar residues, two of which were confirmed by TLC as glucose and rhamnose. Since the ^{13}C NMR spectrum of **1** showed the presence of a carbonyl group consistent with a glucuronic or a galacturonic acid, **1** was converted to its methyl ester (**1a**) by treatment with CH_2N_2. The ^1H and ^{13}C NMR spectra of **1a** shows three anomeric carbon signals at *δ* 101.7, 101.9, and 105.3 and three anomeric proton signals at *δ* 4.98 (d, *J* = 7.2 Hz), 5.86 (d, *J* = 7.7 Hz), and 6.44 (br s). To confirm the identity of the individual sugars and to determine the sequence of the oligosaccharide chain, unambiguous ^1H and ^{13}C NMR assignments were made by combination of the 1D homo-decoupling experiment, NOE difference spectra, $^1H-^1H$ COSY, HMQC, and HMBC. The unknown sugar residue having a carboxyl group (*δ* 170.6) was considered to be a glucuronic acid on the basis of the following data. From HMQC and ^2J and ^3J HMBC experiments, the carbonyl carbon at *δ* 170.6 was assigned to C-6′, and the methine protons at *δ* 4.48 (d, *J* = 8.9 Hz), *δ* 4.30 (t, *J* = 8.9 Hz), *δ* 4.54 (t, *J* = 8.9 Hz), and *δ* 4.51 (dd, *J* = 8.9, 7.2 Hz) were assigned to H-5′, H-4′, H-3′, and H-2′, respectively. In the ^1H NMR, the coupling constants showed *trans*-diaxial relationships between H-1′ and H-2′, H-2′ and H-3′, H-3′ and H-4′, and H-4′ and H-5′. NOESY spectra showed significant through-space interactions between H-1′ and H-3′, H-1′ and H-5′, H-3′ and H-5′, and H-2′ and H-4′ (see Figure 1). The above information suggested this sugar was *β*-D-glucuronopyranose. Since glucose and rhamnose were identified on TLC by acid hydrolysis of **1**, the methylene carbon at *δ* 63.3, having HMQC correlation with *δ* 4.30 and 4.50, was assigned to the C-6″ of the glucose. From $^1H-^1H$ COSY and ^1H homo-decoupling spectra, *δ* 3.85 (m), *δ* 4.06 (t, *J* = 8.8 Hz), and *δ* 4.22 (t, *J* = 8.8 Hz) were assigned to H-5″−H-3″, respectively. In the NOE difference spectra, enhancement of the methine protons at *δ* 3.85 (H-5″) and *δ* 4.22 (H-3″) was observed by irradiation of the anomeric proton at *δ* 5.86 (d, *J* = 7.7 Hz); thus *δ* 5.86 (d, *J* = 7.7 Hz) was assigned to H-1″ of glucose (see Figure 1). Since the coupling was detected at *δ* 4.28 (dd, *J* = 8.8, 7.7 Hz) in the ^1H homo-decoupling spectrum by irradiation at H-1″ (*δ* 5.86), the proton at *δ* 4.28 was assigned to H-2″. The coupling patterns of H-2″ (*δ* 4.28) and H-4″ (*δ* 4.06) suggested *trans*-diaxial relationships between H-1″ and H-2″, H-2″ and H-3″, H-3″ and H-4″, and H-4″ and H-5″. NOESY spectra showed significant through-space interactions between H-1″ and H-3″, H-1″ and H-5″, H-3″ and H-5″, and H-2″ and H-4″ (Figure 1). The above information and HMBC correlations confirmed this sugar was *β*-D-glucopyranose. For the third sugar moiety, the methyl protons at *δ* 1.75 (3H, d, *J* = 6.1 Hz) were considered to be H-6‴ of rhamnose. From $^1H-^1H$ COSY and ^1H homo-decoupling spectra, the methine proton at *δ* 5.02 (m), *δ* 4.35 (t, *J* = 9.2 Hz), *δ* 4.68 (dd *J* = 9.2, 3.4 Hz), *δ* 4.79 (dd, *J* = 3.4, 1.5 Hz), and *δ* 6.44 (d, *J* = 1.5 Hz) were assigned to H-5‴−H-1‴, respectively. In the NOESY spectrum, the methine proton (H-5‴) at *δ* 5.02 (m) showed correlation with the methine proton (H-3‴) at *δ* 4.68 (dd *J* = 9.2, 3.4 Hz). In HMQC spectra, there were cross-peaks between the methyl protons at *δ* 1.75 (H-6‴) and the methyl carbon at *δ* 19.0, the methine proton at *δ* 5.02 (H-5‴) and the methine carbon at *δ* 69.4, and the anomeric proton at *δ* 6.44 and the anomeric carbon at *δ* 101.7. Furthermore, in ^2J and ^3J HMBC experiments, the methyl protons at *δ* 1.75

* To whom correspondence should be addressed. Phone and Fax: (81) 424-95-8912. E-mail: diamonds@my-pharm.ac.jp.
[†] Meiji Pharmaceutical University
[‡] Research Institute of Evolutionary Biology.

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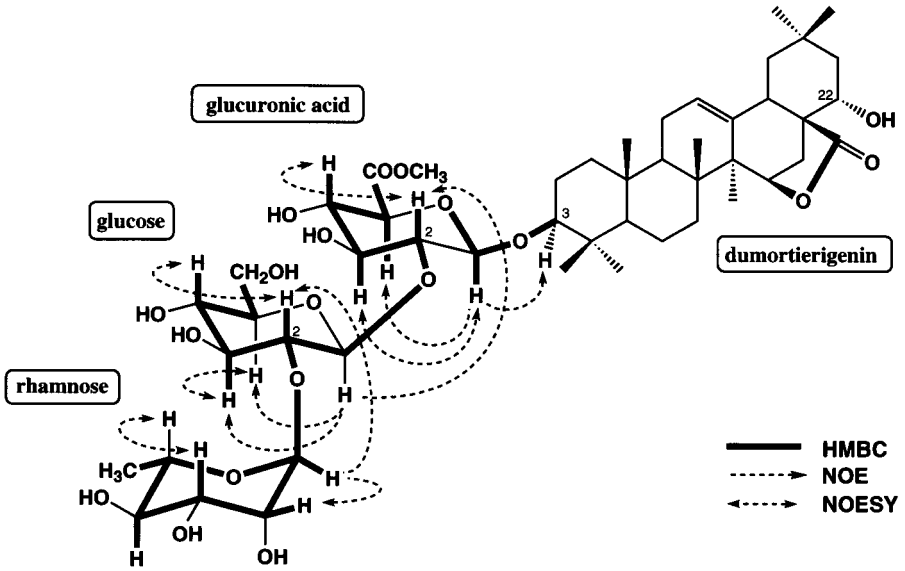

Figure 1. NOE and HMBC of dumortierinoside A methyl ester.

Table 1. ^{13}C and ^{1}H NMR Spectral Data of **1a** in Pyridine-d_5

position	δ ^{13}C	δ ^{1}H	position	δ ^{13}C	δ ^{1}H
1	39.1	1.42 (m)	Glc A		
2	26.4	1.75 (m), 2.05 (m)	1	105.3	4.98 (d, J = 7.2 Hz)
3	89.8	3.30 (dd, J = 12.1, 4.4 Hz)	2	78.3	4.51 (dd, J = 8.9, 7.2 Hz)
4	39.6		3	78.6	4.54 (t, J = 8.9 Hz)
5	56.0	0.81 (br d, J = 9.2 Hz)	4	73.2	4.30 (t, J = 8.9 Hz)
6	18.2	1.37 (m), 1.55 (m)	5	76.8	4.48 (d, J = 9.8 Hz)
7	33.7	1.56 (m)	6	170.6	
8	40.6		COO*Me*	52.0	3.71 (s)
9	48.5	1.55 (m)	Glc		
10	36.8		1	101.9	5.86 (d, J = 7.7 Hz)
11	23.6	1.81 (m)	2	78.4	4.28 (dd, J = 8.8, 7.7 Hz)
12	127.9	5.44 (t-like, J = 3.4 Hz)	3	79.4	4.22 (t, J = 8.8 Hz)
13	138.2		4	72.7	4.06 (t, J = 8.8 Hz)
14	46.6		5	77.8	3.85 (m)
15	80.0	4.64 (d, J = 5.7 Hz)	6	63.3	4.30 (dd, J = 11.3, 2.7 Hz)
16	26.9	2.42 (d, J = 12.4 Hz),			4.50 (dd, J = 11.3, 2.7 Hz)
		2.72 (dd, J = 12.4, 5.7 Hz)	Rha		
17	52.5		1	101.7	6.44 (d, J = 1.5 Hz)
18	42.5	2.65 (br d, J = 14.0 Hz)	2	72.5	4.79 (dd, J = 3.4, 1.5 Hz)
19	46.1	1.56 (m)	3	72.6	4.68 (dd, J = 9.2, 3.4 Hz)
20	31.8		4	74.2	4.35 (t, J = 9.2 Hz)
21	44.9	1.52 (m), 1.88 (m)	5	69.4	5.02 (m)
22	65.3	4.62 (dd, J = 11.3, 5.7 Hz)	6	19.0	1.75 (d, J = 6.1 Hz)
23	28.3	1.34 (s)			
24	16.7	1.06 (s)			
25	16.1	0.81 (s)			
26	19.8	1.21 (s)			
27	25.2	1.23 (s)			
28	179.6				
29	33.0	0.96 (s)			
30	24.9	0.98 (s)			

(H-6‴) and the anomeric proton at δ 6.44 (H-1‴) showed correlation with the methine carbon at δ 69.4. The above evidence suggested this sugar was α-L-rhamnopyranoside. Therefore, the three sugars were confirmed as rhamnose, glucose, and glucuronic acid.

The aglycone portion was identified as dumortierigenin by the HMQC and HMBC correlations of **1a**. Dumortierigenin has two hydroxyl groups at C-3 and C-22, either of which can link with the sugar. The ^{13}C NMR signals due to the sapogenol moiety showed a downfield shift by 11.9 ppm at C-3 (δ 89.8) in comparison with that of dumortierigenin (δ 77.9),[4] but C-22 (δ 80.0) showed no downfield shift. The linkage of the sugar units at C-3 and the sequence of the sugar chains were established by the following combination of HMQC, HMBC, phase-sensitive NOESY, and NOE difference spectra. The ^{2}J and ^{3}J HMBC spectrum of **1a** revealed a cross-peak between the anomeric proton (H-1′) of glucuronic acid at δ 4.98 and the C-3 methine carbon at δ 89.8. In the difference NOE spectrum, enhancement of the methine proton at δ 3.30 (dd, J = 12.4, 4.1 Hz) was observed by irradiation of the anomeric proton (H-1′) of glucuronic acid at δ 4.98. These results suggested the oligosaccharide chain was connected to this position. The negative ion HRFABMS showed fragment peaks at m/z 807 due to ([M−H]-rhamnose), m/z 645 due to ([M−H]-rhamnose-glucose), and m/z 469 due to ([M−H]-rhamnose-glucose-glucuronic acid). The proton (H-2′) of glucuronic acid at δ 4.51 and the proton (H-2″) of glucose at δ 4.30 show HMBC correlations with the anomeric carbons of glucose (δ 101.9) and rhamnose (δ 101.7), respectively. In

addition, NOE enhancements were observed for the H-2′ of glucuronic acid at δ 4.51 and the H-2″ of glucose at δ 4.28, on irradiation of the anomeric protons of glucose at δ 5.86 and rhamnose at δ 6.44, respectively. From the above evidence, the structure of **1** could be elucidated as dumortierigenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranoside.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto MP micromelting point apparatus. The IR spectra were measured with a JASCOA-102 IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using a JEOL GSX-400 (^{1}H 400 and ^{13}C 100 MHz) and a JEOL JNM-LA500 (^{1}H 500 and ^{13}C 125 MHz) spectrometer in pyridine-d_5. Chemical shifts are recorded in ppm (δ) in pyridine-d_5 and referenced to residual solvent peaks at δ 7.20 and δ 135.5, respectively. The $[\alpha]_D$ values were determined with a JASCO DIP-140 digital polarimeter. CC was carried out on 70−230 mesh silica gel (Merck). HPLC was performed using an SSC-3100-J pump with an Oyo-Bunko Uvilog 7 UV detector. The negative ion HRFABMS and EIMS spectra were obtained using a Fisons Analytical VG Autospec.

Plant Material. *I. dumorrtieri* Backbg. (Cactaceae) was cultivated originally at the Research Institute of Evolutionary Biology (Setagaya-ku, Tokyo, Japan), Izu National History Park (Itoh, Shizuoka, Japan), and the Japan Cactus Planning Co. (Fukushima City, Fukushima, Japan). These cacti were identified by Drs. N. Kondo and H. Yuasa. A voucher specimen is deposited at the Research Institute of Evolutionary Biology.

Extraction and Isolation. Dry *I. dumortieri* was extracted with $CHCl_3$ to remove free triterpenes and then repeatedly with MeOH. The MeOH extract (4.23 g) was subjected to column chromatography on Si gel using stepwise gradient ($CHCl_3$−MeOH−H_2O 30:10:0.5 → 30:12:2 → 30:15:3 → 30:20:5) and yielded a white powder, named dumortierinoside A (**1**, 272.9 mg) by precipitation in MeOH. **1** (55 mg) was methylated with diazomethane (CH_2N_2) and purified by column chromatography on Si gel using stepwise gradient ($CHCl_3$−MeOH−H_2O 30:10:0.5 → 30:12:2 → 30:15:3 → 30:20:5) to yield dumortierinoside A methyl ester, **1a** (24.3 mg).

Dumortierinoside A (1): white amorphous powder (272.9 mg); mp >300 °C; $[\alpha]^{20}_D$ −45.41° (*c* 0.011, MeOH); IR ν_{max} (KBr) 3400, 2950, 1770 cm^{-1}; negative ion HRFABMS *m*/*z* 953.4748 ([M−H]$^-$) calcd for $C_{48}H_{74}O_{19}$, 953.4746; negative ion FABMS *m*/*z* 953 ([M−H]$^-$), 807 ([M−H]$^-$ − Rha), 645 (([M−H]$^-$ − Rha-Glc), 469 (aglycone moiety − H).

Dumortierinoside A methyl ester (1a): white amorphous powder, mp 215−218 °C (dec); IR ν_{max} (KBr) 3400, 2950, 1755, 1630, 1385, 1080, 1040 cm^{-1}; ^{1}H and ^{13}C NMR in pyridine-d_6, see Table 1.

Acid Hydrolysis of Dumortierinoside A (1). Compound **1** (33.4 mg) was hydrolyzed with 3.5% HCl (15 mL) at 110 °C for 2.5 h. The $CHCl_3$-soluble fraction was subjected to column chromatography on Si gel and purified by HPLC over Si gel (Nucleosil 50-5, 1 × 25 cm), eluting with $CHCl_3$−MeOH (100:1), resulting in the isolation of the aglycone (9.0 mg), which was identical with dumortierigenin with respect to the TLC and ^{1}H and ^{13}C NMR spectra in pyridine-d_5.

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